Urinary melatonin, LH, oestradiol, progesterone excretion during the menstrual cycle or in women taking oral contraceptives

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Abstract. Nocturnal urinary excretion of melatonin, LH, progesterone and oestradiol was measured by radioimmunoassay in nine normal women during a complete cycle. In addition, these hormonal excretions were studied in two women taking an oral contraceptive. A high within-subject coefficient of variation was observed for melatonin excretion in the two groups. In the nine normal cycling women, melatonin excretion was not decreased at the time of ovulation, but was significantly increased during the luteal phase compared with that of the follicular phase ($P < 0.01$). These data are consistent with a positive relationship between melatonin and progesterone during the luteal phase. In the two women under an oral contraceptive, melatonin excretion was found within the same range as for the other nine. The results are discussed in terms of pineal investigation in human.

The involvement of melatonin – the major indole pineal compound – in the regulation of the hypothalamic-pituitary-ovarian axis in humans remains a point of investigation. In 1976, Wetterberg et al. studying melatonin concentrations in sera sampled in the early morning during the menstrual cycle, showed an elevation of this hormone at the time of menstrual bleeding and a nadir at the time of ovulation.

Melatonin shows a circadian variation with high plasma levels at night but none or only low levels during the day. As a direct relationship between nocturnal plasma melatonin level and melatonin levels in urine collected overnight has been demonstrated for the same subject, measurement of this last parameter appears a good indicator of the total secretion of this hormone by the pineal gland (Lynch et al. 1978). We report here the levels of nocturnal immunoreactive melatonin in urine sampled throughout a complete cycle in women having physiologically regular menses or taking an oral contraceptive. In addition, LH, oestradiol, and progesterone were determined in urine samples as references for the period of the cycle.

Material and Methods

Nine women (mean age ± standard deviation: 32 ± 2 years, range 29 to 36 years) were studied during a complete cycle. They were drug-free and presented a regular menstrual cycle. Six of them had one or several children. In addition, two women (aged 39 and 25 years) taking an oral contraceptive were included in the study. Pills consisted of either a monophasic contraceptive (Stediril®, ethinyl-oestradiol 0.05 mg, norgestrel 0.5 mg) (J) or a biphasic contraceptive (Adepal®, ethinyl-oestradiol 0.03 mg, levonorgestrel 0.15 mg one week, increased to 0.20 mg in the second and third weeks of the cycle) (K).

Urine was collected each night between 19.00 and 07.00 h. During the time of collection, samples of a preceding urination were kept at 4°C. At 07.00 h, each sample was mixed and the volume measured. The month of investigation for each woman is mentioned in Fig. 1.
Urine melatonin was determined using a radioimmunoassay previously described (Claustrat et al. 1984) and validated for urine samples (Brun et al. 1985), and especially, the cross-reactivity of 6-hydroxymelatonin, a major urinary catabolite of melatonin, was less than 0.01%.

Each urine sample was assayed in duplicate and all samples in a cycle were run in the same assay. Results
Fig. 2.
Mean daily ($\pm$ SEM) melatonin and LH levels measured in 9 cycling women. Day zero is the day of the LH peak.

Results
The nocturnal steroid and LH patterns in all the women with a physiological cycle exhibited evidence of the occurrence of ovulation and adequate luteal function. For melatonin excretion, a large intra-individual variation was observed (Fig. 1); the within-subject coefficients of variation (CV) over a complete cycle were between 32.8 and 107.1%. The mean of NUIM concentrations in the nine women was 104.2 $\pm$ 33.3 ng/12 h (extreme values from 43.4 ng/12 h to 156.4 ng/12 h).

The variation in NUIM levels during the 9 normal menstrual cycles aligned on the day of the LH peak is illustrated in Fig. 2. The melatonin level was not significantly decreased at day zero (DO) or D +1 when compared with that of D −1 or D +2. In addition, the lowest melatonin levels were observed on some days of the follicular phase.

Means of NUIM concentrations during the follicular and the luteal periods (93 $\pm$ 18.7 ng/12 h vs 115.4 $\pm$ 19 ng/12 h; mean $\pm$ 1 SD) were significantly different ($P < 0.01$).

In the two women taking an oral contraceptive, the mean levels of melatonin excretion were in the same range as for the other nine: 118 ng/12 h and 152 ng/12 h, respectively. In these controls, despite the absence of LH peak and luteal function, a fluctuation in melatonin excretion was observed (Fig. 3): within-subject coefficients of variation were 37.1% in the woman taking the monophasic pill (J) and 29.8% in the woman taking the biphasic pill (K). In the latter woman, the increase in the progestative dose did not influence the NUIM level.
This study is the first report describing the pattern of urinary melatonin throughout a complete menstrual cycle. Mean levels of NUIM were in the same range as those reported between 19.00 and 07.00 h by Lang et al. (1981) in eleven women, i.e. 18.4–143.4 ng/12 h; mean ± sd = 66.1 ± 31.8 ng/12 h). In their control group, however, neither the genital stage nor the cycle period were indicated.

We observed melatonin levels widely dispersed within a same cycle. On the other hand, the between-subject coefficient of variation was low (CV: 32%). With reference to the methodological aspects in pineal investigation, these data suggest that just one urine sample is not enough to reflect faithfully the pineal secretion in women, although some research has been based upon this approach (Wetterberg et al. 1983). Consequently, repetitive sampling is required or the ovarian cycle phase must be mentioned when melatonin is studied in women.

Considering these large day-to-day variations, one may wonder whether nocturnal urine melatonin levels strictly correlate with plasma melatonin levels sampled at midnight. In such a case, as with the urine level fluctuations, the circadian plasma pattern could be widely dispersed. This last hypothesis does not hold, however, if we make reference to the low variation in the nocturnal plasma pattern observed in the woman at different periods of the menstrual cycle (Birau 1981; Hariharasubramanian et al. 1985).

We were not able to detect any decrease in melatonin excretion at the time of ovulation. In comparison with the follicular phase, the luteal phase showed a significant increase in the NUIM level. Our results are in concordance with the positive relationship between the circadian pattern of melatonin and progesterone reported by Webley et al. (1986) and support the idea that the rise in melatonin secretion during the luteal phase could reinforce the steroidogenesis by the corpus luteum, in agreement with the report that melatonin directly stimulates the in vitro progesterone production by human granulosa cells (Mac Phee et al. 1975). Further, the melatonin pattern we have described during the menstrual cycle provides a supplementary physiological validation of our radioimmunoassay applied to urine samples.

In the women taking an oral contraceptive, no
conclusion could be drawn because of the too small sample. However, it should be mentioned that although the two contraceptive pills were different, the treatment in both cases included a continuous administration of progestogen during a complete cycle.

In conclusion, we have defined reference values in well-controlled hormonal status conditions using a non-invasive methodology which allows frequent sampling in female outpatients, without the assistance of medical or paramedical personnel. Such an approach is of great interest as it can be extended to pathological groups for longitudinal studies.

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